December 5, 2007.

To : Dr. Yelena G. Gakh
US patent & Trademark Office
PO Box 1450
Alexandria, VA 22313-1450

Re : Application No. 10/675,764
Art Unit 1743

Dear Dr. Yelena G. Gakh :

We, the applicants, would like to reply to the office action on 09-25-2007 as follows :

1. Office action : Claims 25-44 are pending in the application. Claims 30, 32 and 36 are withdrawn from consideration as directed toward non-elected species. Claims 25-29, 31, 33-35 and 37-44 are considered on merits.

   Reply : We acknowledge this office action and do not amend claims 30, 32, and 36.

2. Office action : The information disclosure statement filed 09/30/03 fails to comply with 37 CFR 1.98(a)(2), which requires a legible copy of each cited foreign patent document; each non-patent literature publication or that portion which caused it to be listed. It has been placed in the application file, but the information referred to therein has not been considered.

   Reply : We attach here all non-patent publications cited in the references. One non-patent publication by Peter Husek, titled "Chloroformates in gas chromatography as general purpose derivatizing agents", Journal of Chromatography B, 1998, page 57-91, vol. 717 is not submitted because the office sent a copy to us.

3. Office action : The subject matter of this application admits of illustration by a drawing to facilitate understanding of the invention. Applicant is required to furnish a drawing under 37 CFR 1.8 1 (c). No new matter may be introduced in the required drawing. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). At least one mass spectrum of the carboxylic acid ester and internal standard should be provided for illustrative purposes.

   Reply : We would like to submit drawings which include mass spectrum of the labeled carboxylic acid ester internal standard (ketoprofen ethyl ester-d5), mass spectrum of derivatized carboxylic acid (ketoprofen ethyl ester), mass spectrum of a typical MS analysis of a sample containing both ketoprofen ethyl ester-d5 and ketoprofen ethyl ester in MRM mode (multiple reaction monitoring). A new part, brief description of the drawings, will be added to the specification to explain the figures.

4. Office action : Claims 41,42 and 44 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 41 does not recite any active step and therefore does not further limit the subject matter of the parent claim. Regarding claim 43, all steps in parent claim 25 are obviously performed in the sequence recited in the claim, and therefore claim 43 does not further limit the subject matter of claim 25. Regarding claim 44, claim 25 recites specific reactions for the conversion step, which are quantitative, and therefore claim 44 does not further limit the subject matter of claim 44.

Reply : We would like to cancel claim 41 because it recites matter already claimed in claim 25. The applicants also would like to cancel claim 43 and claim 44 for the same reason.

5 . Office action : The following is a quotation of the first paragraph of 35 U.S.C. 112: The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 25-29, 31, 33-35 and 37-44 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for method, in which a carboxylic acid ester internal standard is an ester of the carboxylic acid to be identified, does not reasonably provide enablement for the method, in which the internal standard has a different structure. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.
Claim 25 recites that the carboxylic ester obtained from the carboxylic acid should be of an identical structure as that of the internal standard, which is possible only if the internal standard is the ester of the carboxylic acid to be identified. On the other hand, if the internal standard has a known structure, and the structure is the same as that of the ester of the carboxylic acid to be identified, it is not clear, why the carboxylic acid should be identified at all, since its structure is a priori known.

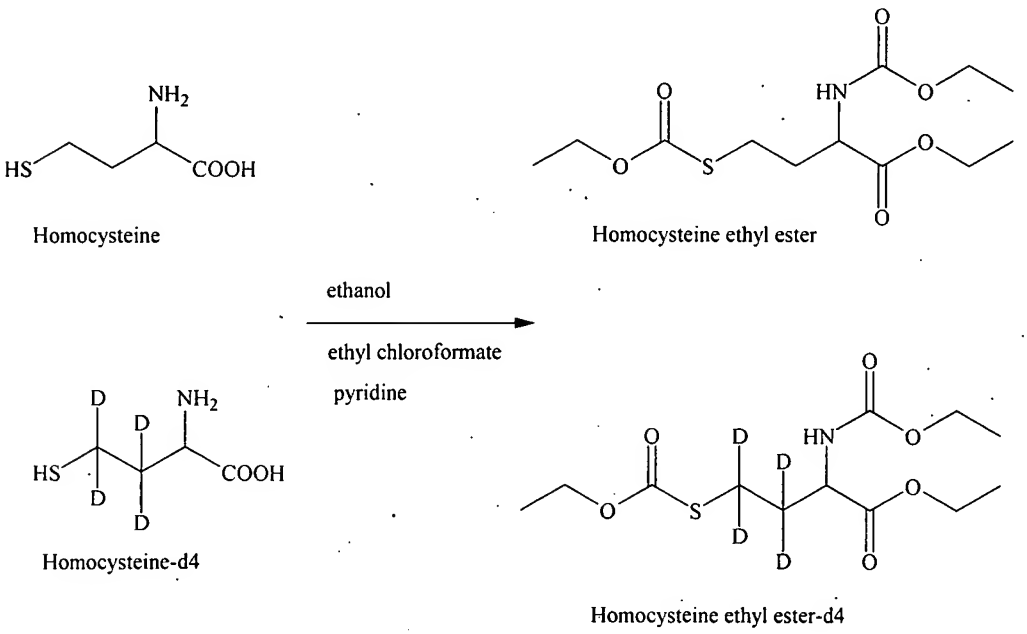
Reply : We might have mislead you by the language of the previous specification and the claims. We now ask your permission to amend the specification and the claims with "rephrasing" and "rewritten" sentences to clarify the subject matter without introducing new matter.

The invention provides a method of identification and quantification of carboxylic acid in a sample by MS analysis by synthesizing labeled compound that can be used as labeled internal standard. The use of the synthesized labeled compound as labeled internal standard requires that the carboxylic acid in the sample be converted to a compound with identical chemical structure as the synthesized labeled internal

standard. The subject matter of the invention can be clarified by rephrasing as "methods of one step synthesis of labeled internal standards and additional reaction for the purpose of identification and quantification of carboxylic acid in a sample by mass spectrometry analysis...". This rephrasing does not introduce new matter because the specification contains disclosure that supports the synthesis of labeled internal standard and the additional reaction for the MS analysis of carboxylic acid. These reactions are one step reactions that make the MS analysis of carboxylic acid novel. The language as written before might have led the office to the understanding that the invention is the MS analysis of the carboxylic acid.

So the subject matter of the invention is actually "a method of one step synthesis of stable isotope labeled internal standard and additional chemical reaction for the purpose of identification and quantification of carboxylic acid in an aqueous sample by mass spectrometry analysis". It is these chemical reactions of carboxylic acid that form the novelty of the invention. At this point we would like to use the MS analysis of homocysteine in the cited reference of Jens Pietzsch et al. to clarify the confusion about the analysis of the carboxylic acid using the internal standard having different chemical structure.

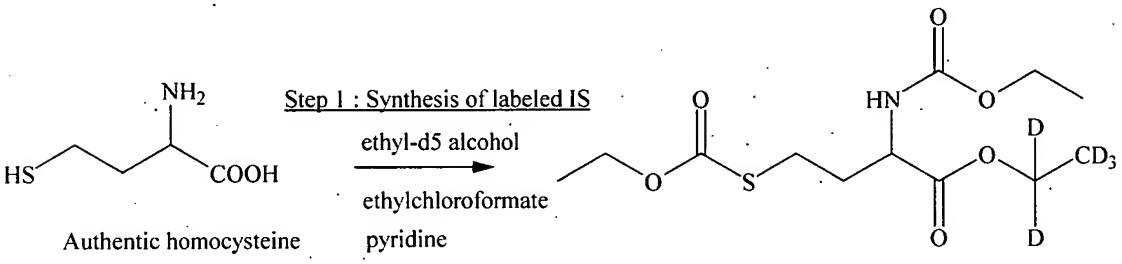
Homocysteine is a carboxylic acid because of the presence of a carboxyl functional group. In the analysis of homocysteine in a patient sample by gas chromatography-mass spectrometry, homocysteine-d4 was used as a labeled internal standard. A sample containing homocysteine was processed by addition of a known amount of homocysteine-d4. The resulting sample was treated with a derivatizing reagent consisting of ethyl alcohol, ethyl chloroformate, and pyridine. Homocysteine in the sample was derivatized or converted to homocysteine ethyl ester while homocysteine-d4 was converted to homocysteine ethyl ester-d4. Structures of both esters are shown below.



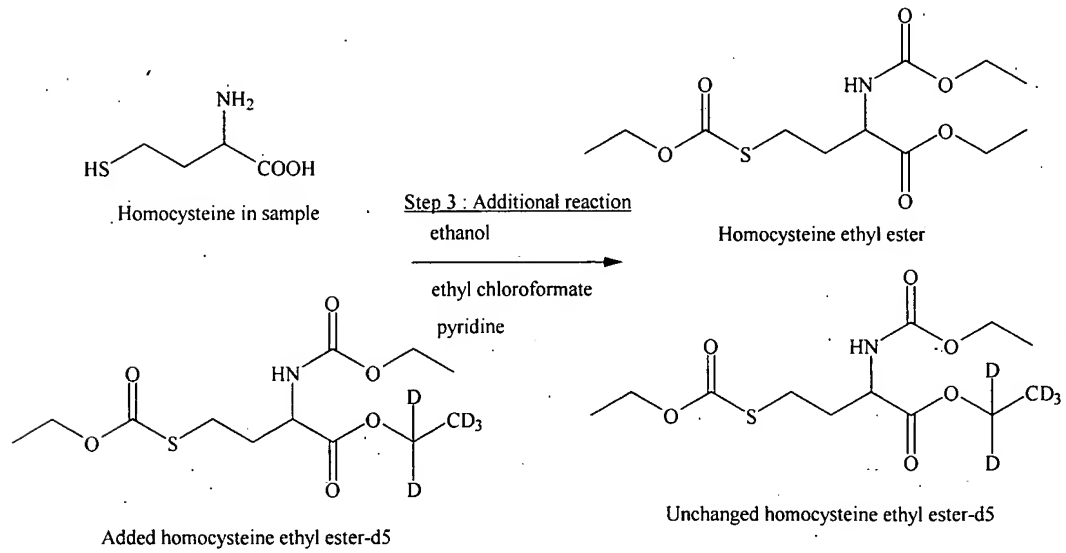
Jens Pietzsch et al.

Upon MS analysis of the extract containing homocysteine ethyl ester and homocysteine ethyl ester-d4, the ion signals of both esters are obtained and the concentration of homocysteine is deduced from the calibration curve of homocysteine ethyl ester. The carboxylic acid homocysteine is not identified at all, but it is identified as homocysteine ethyl ester and its concentration is obtained from an analysis of this ethyl ester. With the exception of the labeled isotope atoms, both homocysteine ethyl ester and homocysteine ethyl ester-d4, the labeled internal standard, have identical chemical structure. This is the requirement of MS analysis using labeled internal standard.

The methods of our invention enable people skilled in the art to practice MS analysis in cases where labeled internal standard is not available. How do people skilled in the art practice our methods in the analysis of homocysteine when homocysteine-d4 is not available.



Homocysteine ethyl ester-d5



Homocysteine ethyl ester

Added homocysteine ethyl ester-d5

Unchanged homocysteine ethyl ester-d5

Refer to the above drawings, first, Step 1, homocysteine ethyl ester-d5 is synthesized in one step by reaction of an authentic sample of homocysteine and ethyl-d5 alcohol in the presence of a base and a chloroformate.

Second, the synthesized homocysteine ethyl ester-d5 is used as labeled internal standard and is added in known amount to a sample containing homocysteine.

Third, step 3, the resulting sample now contained homocysteine ethyl ester-d5 and homocysteine is treated with ethyl alcohol in the presence of a base and a chloroformate to convert homocysteine in sample into homocysteine ethyl ester while homocysteine ethyl ester-d5 in the sample is not reacting at all.

Fourth, the sample is processed by an aqueous extraction to isolate homocysteine ethyl ester and homocysteine ethyl ester-d5.

Fifth, MS analysis of the extract provides concentration of the homocysteine ethyl ester.

Sixth, concentration of homocysteine in the sample is deduced from the calibration curve made up of samples of known concentration of homocysteine and are processed the same way.

Both homocysteine ethyl ester and homocysteine ethyl ester-d5 in the final extract have the identical chemical structure with the exception of the labeled isotope atoms and this fulfills the requirement for MS analysis. The people skilled in the art can recognize the novelty of the synthesis of homocysteine ethyl ester-d5. It is not known in prior art that this method is used for such synthesis of labeled internal standard for the MS analysis of carboxylic acid. The requirement for identical chemical structure is fulfilled by the additional reaction of homocysteine, the conversion of homocysteine in the sample to homocysteine ethyl ester in the presence of homocysteine ethyl ester-d5. The combination of one step synthesis of labeled compound that can be used as labeled internal standard and the additional reaction of the sample containing the compound to be analyzed is the subject matter of the invention. This combination makes the MS analysis of carboxylic acid novel.

6. Office action : Claims 25-29,31,33-35,37-40 and 42-44 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for method, "wherein there is not conversion of the stable isotope labeled carboxylic acid ester internal standard to its corresponding non-labeled carboxylic acid ester compound during step b)", as recited in claim 41, does not reasonably provide enablement for the method, for which such requirement is not fulfilled.

   Reply : We ask that the phrase from claim 25 "wherein there is not conversion of the stable isotope labeled carboxylic acid ester internal standard to its corresponding non-labeled carboxylic acid ester compound during step b)" be amended as "wherein there is no reaction of said labeled carboxylic acid ester internal standard with said non labeled derivatizing reagent" to clarify that the non labeled derivatizing reagent only reacts with carboxylic acid in the sample but not with the added labeled carboxylic acid ester internal standard.

7. Office action : The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 25-29, 31, 33-35 and 37-44 are rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 25 recites converting the carboxylic acid in said sample into a "carboxylic acid ester of identical structure as that of said carboxylic acid ester internal standard except for the stable isotope atoms". First, it is not clear, why the carboxylic acid should be identified, if the internal standard is of the same structure as the ester of the carboxylic acids that is supposed to be identified? There is a contradiction in the subject matter of the claim, which renders claim 25 and all dependent claim unclear. Further, it is not clear, which "stable isotope atoms" are meant in the claim, and which compound they belong to – the internal standard, or the carboxylic acid? This uncertainty renders claims 25 and 27 unclear and indefinite. It is not apparent, if for the plurality of carboxylic acids to be identified a plurality of internal standards with a priori known structures should be prepared. Also, if it would be possible to prepare a plurality of internal standard with known structures, it means that all carboxylic structures are known, and therefore it is not clear, what is the purpose of the method. Just quantifying of the known carboxylic acids? The subject matter of the pending claims should be clarified. Claim 41 is not clear. Which conditions does it recite in order to fulfill the requirement of the recitation? The condition does not seem to be recited. Moreover, the requirement recited in claim 41 should be inherent for the method recited in the parent claim; otherwise, the method for quantification of the carboxylic acid recited in claim 25 becomes un-enabled.

Reply : With the permission for amendment of the specification and the claims, you can see that the invention provides methods of MS analysis of carboxylic acid in a sample as its carboxylic acid ester. The desired concentration of the carboxylic acid is provided by the analysis of the carboxylic acid ester. If you refer back to the example of MS analysis of homocysteine using our methods in answer 5 above, the analysis of homocysteine ethyl ester in the extract provides the concentration of homocysteine in the sample. The use of the synthesized homocysteine ethyl ester-d5 requires that homocysteine in the sample be converted to homocysteine ethyl ester before MS analysis. This conversion reaction is the additional reaction that fulfills the requirement of "identical chemical structure, except the labeled isotope atoms"of both homocysteine ethyl ester and homocysteine ethyl ester-d5. We ask your permission to amend the specification and the claims to add claim 44 to clarify the stable isotope atoms which include deuterium, carbon-13, nitrogen-15, and oxygen-18.

The synthesis of labeled chemical compounds provided by the methods of our invention is a one step reaction using labeled alcohol (ie. Ethyl-d5 alcohol). The additional reaction of the sample to convert the compound being analyzed (ie. homocysteine) to compound of the same chemical structure as that of the labeled internal standard, except the labeled atoms (ie. homocysteine ethyl ester) is also a one step reaction. The advantage of the methods of our invention is that because the

labeled derivatizing reagent, ethyl-d5 alcohol, is the reagent that chemically attaches a labeled ethyl group to the carboxyl function of the carboxylic acid, this reaction can be performed on a mixture of more than one carboxylic acid. The result is a simultaneous and one step synthesis of labeled internal standards that is not known in prior art. It is not known in prior art because the use of these synthesized labeled internal standards requires additional reaction to be performed on the sample containing the mixture of carboxylic acids to convert them to compounds of identical chemical structures as those of the labeled internal standards.

The subject matter of the invention can be further clarified by another distinct advantage, the ability to synthesize labeled internal standards by those who perform the MS analysis. In the MS analysis of homocysteine by Jens Pietzsc et al., homocysteine-d4 has to be independently synthesized, certainly not by those who perform the MS analysis. If there is a desire to analyze another carboxylic acid using similar method by Jens Pietzsch et al., another deuterated carboxylic acid has to be independently synthesized, unlikely by those who perform the MS analysis. The methods of our invention allows the same one step reaction with the same reaction conditions for both the synthesis of labeled internal standard and the additional reaction. The former reaction uses a labeled derivatizing reagent while the latter reaction uses the same but non labeled derivatizing reagent.

8. Office action : Claim Rejections - 35 USC 8 103
The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:
(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in Graham v. John Deere Co., 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the

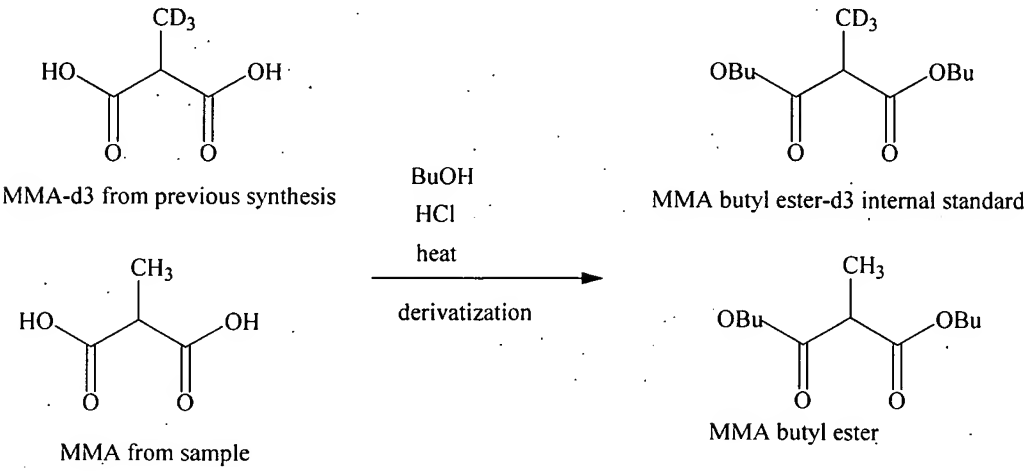
examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 25-29,31,33-35 and 37-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Magera et al. (Clin. Chem., 2000) in view of HuSek (J. Chromat. By 1998).
Magera does not specifically teach adding internal standard in the form of ester or preparing butyl or other alkyl esters using chloroformate and corresponding alcohol.

HuSek indicates that chloroformates were revealed "as extraordinarily suitable esterification agents for transforming the carboxyl into the ester instantaneously" (using corresponding alcohols) (page 67).

It would have been obvious for any person of ordinary skill in the art to prepare esters disclosed by Magera using HuSek's method, because HuSek specifically indicates that chloroformates are extraordinary suitable agents for transforming the carboxyl into the ester instantaneously. It would have been obvious for any person of ordinary skill in the art to first prepare isotopically labeled internal standards in the form of deuterated esters and add them to the sample comprising carboxylic acid(s), because the esters can be prepared by the same efficient esterification method that is taught by HuSek using relatively cheap deuterated alcohols.

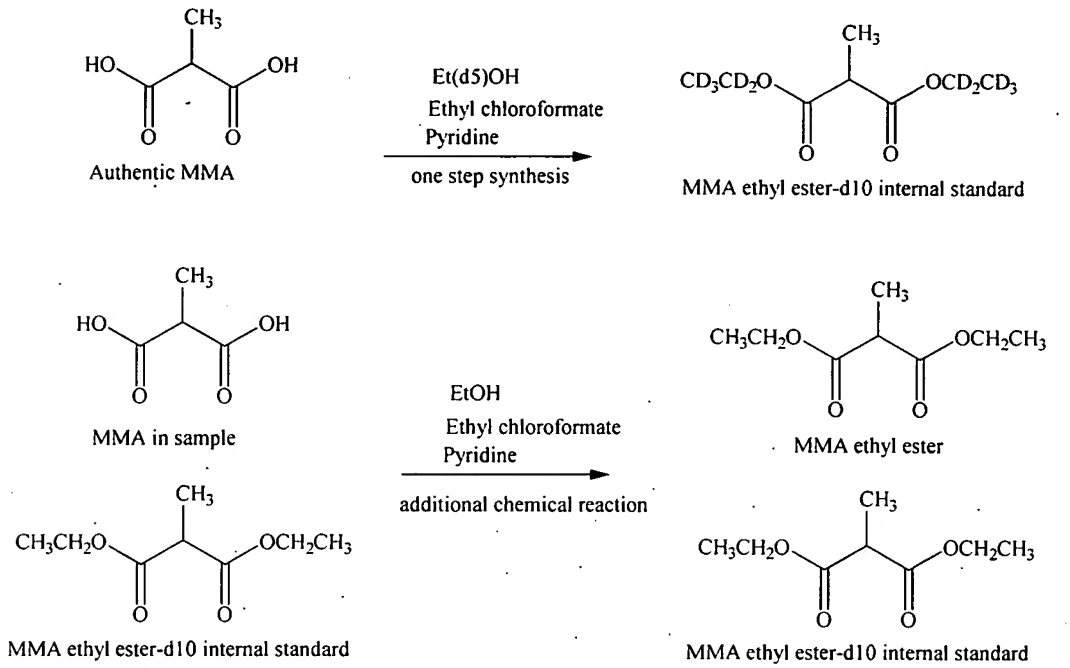
Reply : Magera et al. teach method of measuring methylmalonic acid (MMA) in plasma and in urine sample by stable-isotope dilution and electrospray tandem mass spectrometry using MMA-d3 as labeled internal standard. After addition of a known amount of MMA-d3 to a sample, both MMA and MMA-d3 are derivatized to the butyl ester derivatives whose chemical structures are shown below.



Method of Magera et al.

The method of Magera et al. relies on a supply of MMA-d3 which could have been synthesized by a multi step synthesis. Using the method of our invention, the

supply of MMA-d3 is not required. Instead MMA ethyl ester-d10 can be synthesized by a one step synthesis from MMA, the readily available ethyl-d5 alcohol, ethyl chloroformate, and pyridine. The use of MMA ethyl ester-d10 requires an additional reaction to be performed on MMA, the conversion of MMA to the chemical compound of identical chemical structure as MMA ethyl ester-d10, except the deuterium atoms as shown.



Method of our invention

The method of our invention takes advantage of a reaction of carboxylic acid and a labeled ethyl-d5 alcohol to introduce a labeled alkyl group ethyl-d5 to the resulting ester. Even though Husek teaches methods of derivatization of carboxylic acid to carboxylic acid ester, it is not anticipated from Magera's work that MMA ethyl ester-d10 can be prepared by Magera et al. for the analysis.

A labeled chemical compound that is useful for MS analysis purpose must have at least 2 stable isotope atoms, preferably carbon-13 because of its very low isotopic abundance in naturally occurring carbon. The synthesis of MMA labeled with at least 2 carbon-13 is a difficult synthesis involving multi step reactions. However the synthesis of carbon-13 labeled compound that can be used as labeled internal standard for the analysis of MMA can be accomplished by the use of ethyl alcohol $^{13}C_2$ having both carbon-13 as the labeled derivatizing reagent, the methods of our invention. The one step synthesis of carbon-13 labeled internal standard is not anticipated from Magera et al.'s work.

This one step synthesis of carbon-13 labeled internal standard also find itself advantageous in the MS analysis of carboxylic acid such as formic acid in which there is only one possible carbon-13 labeled formic acid (formic acid $^{13}C_1$). The

methods of our invention make possible the synthesis of formic acid ethyl ester $^{13}C_2$ that can be used for the MS analysis of formic acid.

It is a fact that HuSek teaches method of derivatization of carboxylic acid to carboxylic acid ester for gas chromatographic analysis and certainly suitable for mass spectrometry analysis. However, it is not anticipated from HuSek's work that labeled internal standards, especially for carboxylic acids, are prepared using labeled derivatizing reagent. In fact there was no example of this type of synthesis found in all publications of Husek or others. So, in establishing a background for determining obviousness under 35 U.S.C. 103(a) the above discussion can summarized as follows:

1. The scope of prior art is the derivatization of carboxylic acid including labeled carboxylic acid to carboxylic acid esters while the methods of the present invention "expand the application" of the derivatization to the synthesis of labeled compounds that can be used as labeled internal standards.
2. The differences between prior art and present methods of the invention are advantages of the methods of the invention as mentioned above such as a) the ability to synthesize labeled internal standards by those who perform the analysis; b) the synthesis is always one step reaction; c) the synthesis of more than one carboxylic acid esters can be performed simultaneously using a single derivatizing reagent; d) the synthesis of carbon-13 labeled ester is also one step synthesis that can allow the labeling of 2 or more carbon13 atoms; e) the synthesis allows facile synthesis of carbon-13 and deuterium labeled internal standard in cases where both carbon-13 and deuterium labeled carboxylic acid cannot be practically synthesized such ac formic acid.
3. For those skilled in the art, the methods of our invention demonstrate a clear economic advantage for the synthesis of labeled carboxylic acid esters that can be used as labeled internal standards. The objective evidence presented in the specification and the claims indicates a nonobviousness.

We made our best effort to comply with the rules of the patent office in amending the specification and the claims for the purpose of clarifying the subject matter of our invention. There may still be errors both in written requirement and in interpreting the patent rules. We ask for your continued advice and appreciate your past support and patience.

Sinverely and kind regards,

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